Claims

1.   A method to prepare an isolated nucleic acid molecule comprising a
nucleotide sequence encoding at least one of the variable regions of the α and β chains of
5   a non-human TCR which TCR is human HLA-restricted and specific for a tumor-
associated antigen, which method comprises

cloning or amplifying a nucleic acid molecule containing said encoding
nucleotide sequence from cytotoxic T lymphocytes (CTL) prepared by a method which
comprises

10   immunizing a transgenic non-human vertebrate which is modified so as to express
at least one human HLA antigen with said tumor-associated antigen (TAA) so as to effect
the production in said mouse of cytotoxic T lymphocytes which display human HLA-
restricted TCR specific for said TAA and which contain nucleic acid molecules
comprising nucleotide sequences encoding said variable regions of the α and β chains of
15   said TCR, and

recovering said CTL.

2.   The method of claim 1 wherein said HLA antigen is a A2.

20   3.   The method of claim 1 wherein said non-human vertebrate is a mouse.

4.   The method of claim 3 wherein said amplifying is effected by a
polymerase chain reaction using primers derived from murine TCR.

25   5.   The method of claim 4 wherein said primers are essentially as set forth in
Figure 6.

6.   An isolated nucleic acid molecule which comprises a nucleotide sequence encoding a variable region of a non-human TCR α or β peptide wherein said TCR is human HLA-restricted and specific for a tumor-associated antigen.

7.   The nucleic acid molecule of claim 6 which comprises the α or β variable region of the said TCR fused to the ζ region of CD3, CD8 or CD16.

8.   The nucleic acid molecule of claim 7 wherein said ζ region is that of human CD3, CD8 or CD16.

9.   The nucleic acid molecule wherein said non-human TCR is murine.

10.   The nucleic acid molecule of claim 6 wherein said nucleotide sequence encodes a single-chain TCR.

11.   The nucleic acid molecule of claim 10 wherein said single-chain TCR consists of the variable α region fused to variable β region by a flexible linker and said β region is fused to a ζ region.

12.   The nucleic acid molecule of claim 11 wherein said flexible linker is of the formula $(Gly_4Ser_3)_3$.

13.   The nucleic acid molecule of claim 11 wherein said ζ chain is that of CD3, CD8 or CD16.

14.   The nucleic acid molecule of claim 13 wherein the ζ chain is derived from human CD3, CD8 or CD16.

15. A recombinant expression system which expression system comprises the nucleotide sequence of claim 6 operatively linked to control sequences for effecting its expression in a host cell.

5   16. A recombinant host cell modified to contain the expression system of claim 15.

17. The recombinant cells of claim 16 which are T cells.

10   18. A method to obtain cells which display TCR or a functional derivative thereof at their surface, said TCR or derivative being human HLA-restricted and specific for a tumor-associated antigen, which method comprises culturing the cells of claim 16 under conditions wherein said nucleotide sequence is expressed and said TCR or derivative is displayed at the surface.

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19. Recombinant cells displaying a TCR receptor or derivative thereof at their surface wherein said TCR or derivative is human HLA-restricted and specific for a tumor-associated antigen prepared by the method of claim 18.

20   20. A method to identify antigens associated with a tumor which method comprises contacting said tumor or a fraction thereof with the cells of claim 19 under conditions wherein said tumor or fraction is lysed only if said tumor displays the TAA for which said TCR or derivative is specific.

25   21. A method to effect treatment of a tumor in a human, wherein said tumor is characterized by a specific tumor-associated antigen (TAA) which method comprises administering to said human subject peripheral blood cells from said subject which have been modified to contain an expression system for a nucleotide sequence which encodes a TCR or derivative thereof which is human HLA-restricted and specific for said TAA.